



⑯ Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Publication number:

O 132 919
B1

⑫

EUROPEAN PATENT SPECIFICATION

⑯ Date of publication of the patent specification:
29.10.86

⑮ Int. Cl.: **C 07 K 1/00, A 61 K 37/02,**
A 61 K 49/00

⑯ Application number: 84303471.1

⑯ Date of filing: 22.05.84

⑯ Peptides.

⑯ Priority: 31.05.83 JP 97718/83
04.11.83 JP 208066/83

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⑯ Date of publication of application:
13.02.85 Bulletin 85/7

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⑯ Publication of the grant of the patent:
29.10.86 Bulletin 86/44

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⑯ Designated Contracting States:
CH DE FR GB IT LI NL SE

⑯ References cited:
DE - A - 1 800 129
DE - A - 2 256 445
DE - A - 2 751 026

EP O 132 919 B1

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Description

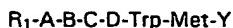
This invention relates to novel peptides.

As regards peptides analogues to the peptide of the invention, there is known for example a peptide represented by formula



(hereinafter this peptide is referred to as «CCK-8»). CCK-8 is known to have a wide variety of physiological actions such as the contraction of gallbladder, stimulation of pancreatic enzyme secretion, stimulation of pancreatic internal secretion, potentiation of intestinal movement, depression of gastric secretion, pancreas proliferation, depression of central nervous system, sedation, enhancement of sleeping time, anti-psychopathia action, anti convulsive action, analgesia, and anorexia.

It is an object of the present invention to provide novel peptides of the following formula (1) and pharmacologically acceptable salts thereof, and a process by which they can be obtained in a high yield and high purity,



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOCCO-; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr(SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or HOOCCO-, B is

Tyr(SO₃H), C is Met, and D is Gly.

It is another object of the present invention to provide a pharmaceutical composition useful for accelerating the pancreatic function, which comprises an effective amount of formula (1) or pharmacologically acceptable salts thereof.

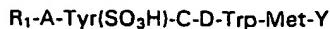
According to one aspect of the present invention, there is provided novel peptides represented by the following formula



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOCCO-; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr(SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or HOOCCO-, B is

Tyr(SO₃H), C is Met, and D is Gly, and pharmacologically acceptable salts thereof.

According to another aspect of the present invention, there is provided a process for the preparation of novel peptides represented by the following formula



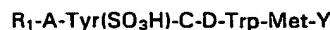
wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOCCO-; A denotes

Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or HOOCCO-, C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that the Tyr-residue in the peptide of the following formula



wherein; R₁, A, C, D, and Y are the same as defined above, or the peptide in which active groups such as amino groups are protected by suitable protecting groups is subjected to the sulfate-ester reaction to convert the Tyr-residue to Tyr(SO₃H) residue.

According to further an aspect of the present invention, there is provided a process for the preparation of novel peptides represented by the following formula



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOCCO-; A denotes

Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or HOOCCO-, C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that a sulfo group of a protected peptide amide sulfate ester obtained by the sulfate-ester reaction of the Tyr residue in the protected peptide of the following formula



wherein; R₁, A, C, D, and Y are the same as defined above, is converted to a salt of divalent metals such as Ca, Zn, and the like to stabilize said protected peptide amide sulfate ester, and thereafter said protected peptide amide sulfate ester is deprotected.

According to still further an aspect of the present invention, there is provided a process for the pre-

paration of novel peptides represented by the following formula



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or  CO-; A denotes

Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp, and pharmacologically acceptable salts thereof, characterized in that a divalent metal salt of a sulfonated p-amino phenylalanine derivative is prepared, and a peptide chain is elongated by use of said divalent metal salt.

According to still further an aspect of the present invention, there is provided an accelerator for pancreatic function which contains a novel peptide represented by the following formula



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or  CO-; A denotes

Asp, Ala, or merely a chemical bond; B denotes Tyr(SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or  CO-, B is

Tyr(SO₃H), C is Met, and D is Gly, and pharmacologically acceptable salts thereof, as an effective component.

According to still further an aspect of the present invention, there is provided a reagent for pancreatic function tests or a contrast medium for gallbladder which contains a novel peptide represented by the following formula



wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or  CO-; A denotes

Asp, Ala, or merely a chemical bond; B denotes Tyr(SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or  CO-, B is

Tyr(SO₃H), C is Met, and D is Gly, and pharmacologically acceptable salts thereof as an effective component.

Brief description of the drawings

Figs. 1 and 2 show pancreatic secretion accelerating activities of various peptides of the pres-

ent invention compared with that of CCK-8. Figs. 3 and 4 show whole protein secretion accelerating activities of those peptides compared with that of CCK-8.

Detailed description of the preferred embodiments

The peptide of the present invention is novel and represented by the general formula

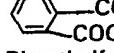


wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene, or  CO-; A denotes Asp, Ala, or merely

a chemical bond (this means that R₁ combines directly with B); B denotes Tyr (SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond (this means that B combines directly with D); D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO- or  CO-, B is Tyr(SO₃H), C is Met, and

D is Gly.

In the present specification, the abbreviations of amino acids, peptides, protective groups, active groups, and other compounds and groups are in accordance with those defined in IUPAC or IUB or with those customarily used in the art. Examples of the abbreviations are given below. Unless otherwise specified, compounds such as amino acids, when they can involve optical isomers, refer to L-isomers thereof.

| | |
|-------|---|
| Asp | Aspartic acid residue |
| Ala | Alanine residue |
| β-Ala | β-Alanine residue |
| Leu | Leucine residue |
| Gly | Glycine residue |
| Met | Methionine residue |
| pGlu | Pyroglutamic acid residue |
| Phe | Phenylalanine residue |
| Trp | Tryptophan residue |
| Tyr | Tyrosine residue |
| Bz | Benzyl group |
| Boc | tert-Butyloxycarbonyl group |
| Z | Benzoyloxycarbonyl group |
| OSu | Succinimidoxyl group |
| Suc | HOOC-(CH ₂) ₂ -CO- |
| Glt | HOOC-(CH ₂) ₃ -CO- |
| Pht |  COOH |
| DMF | Dimethylformamide |
| THF | Tetrahydrofuran |

The peptide of the general formula (1) can be prepared by common methods applied for the synthesis of peptides, for example, methods described in «The Peptides», Vol. 1 (1966) (by Schroder and Luhke, published by Academic Press, New York, U.S.A.) and «Peptide Synthesis» (1975) (by Izumiya et al., published by Maruzen Co., Ltd.). These processes, applicable in the invention, include the azide method, acid chloride method, acid anhydride method, mixed anhydride method, DCC

method, active ester method (p-nitrophenyl) method, N-hydroxysuccimide ester method, cyanomethyl ester method, or the like, the method employing Woodward's reagent K, carbodiimidazole method, oxidation-reduction method, DCC-additive method (HONB, HOBT, HOSu), and solid phase method, etc.

Usually, the peptide of the general formula (1) is prepared according to the above-mentioned general method for the synthesis of polypeptides, for instance, the so-called stepwise process which comprises successive condensations of individual amino acids with a terminal amino acid or the process which comprises coupling reactions of a few fragments to complete the intended polypeptide. More particularly, the present peptide can be prepared by the condensation of a reactive-carboxyl-containing compound with a reactive-amino-containing compound in the usual way, the former compound corresponding to one of the two moieties into which the peptide can be divided by cutting its main chain at an arbitrary position and the latter compound corresponding to the other moiety. When the condensation product has protective groups, the preparation is possible through eliminating the protective groups in the usual way. Aspartic acid, when used in the reaction process for synthesizing the peptide of the formula (1) is desired in most cases to be protected previously. In the final step of the reaction process, all the protective groups are usually eliminated from the peptide in which one or more of the constituent amino acid residues are protected.

In the reaction process for synthesizing the peptide of the formula (1), the functional groups which should not participate in the reaction are also protected by common protective groups, which are eliminated after completion of the reaction. Moreover, the functional groups to participate in the reaction are activated in general.

The above reaction methods are known and reagents used therein can be suitably selected from known compounds.

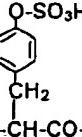
Protective groups for the amino group include, for example, carbobenzoxy (hereinafter abbreviated as Z), tert-butyloxycarbonyl (hereinafter abbreviated as Boc), tert-amyoxy carbonyl, isobornyloxy carbonyl, p-methoxybenzyloxy carbonyl, 2-chloro-benzyloxy carbonyl, adamantlyloxy carbonyl, trifluoroacetyl, phthalyl, formyl, o-nitrophenylsulphenyl, and diphenylphosphinothioyl, etc. Protective groups for the carboxyl group include, for example, alkyl esters (e.g. esters of methyl, ethyl, propyl, butyl, and tert-butyl), benzyl ester, p-nitrobenzyl ester, p-chlorobenzyl ester, benzhydryl ester, carbobenzoxyhydrazide, tert-butyloxycarbonylyhydrazide, and tritylhydrazide, etc.

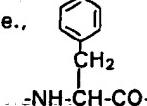
Activated derivatives of the carboxyl group include, for example, acid chloride, acid anhydride or mixed anhydride, azides, and activated esters (esters of pentachlorophenol, p-nitrophenol, N-hydroxysuccinimide, N-hydroxybenzotriazole, and N-hydroxy-5-norbornene-2,3-dicarboxyimide, and the like). Sometimes the peptide-linkage forming reaction can be carried out in the presence of a condensing agent such as a carbodiimide reagent

(e.g., dicyclohexylcarbodiimide or carbodiimidazole) or tetraethyl pyrophosphate, etc.

In the above general formula (1), when R₁ is X-R₂-CO- (X denotes carboxyl or amino and R₂ denotes lower alkylene), R₂ denotes a C₁-C₆ alkylene. In consequence, R₁ in this case denotes succinyl, glutaryl, maleyl, phthalyl, glycyl, β-alanyl, γ-aminobutyryl, pyroglutaminyl, or the like. When R₁ is HOOCCO-, the carboxyl group can be attached to the benzene ring at any of the o-, m-, and p-positions.

The peptide of the formula (1) wherein B is Tyr



(SO₃H), i.e., 

, is favorably prepared in

the following way:

A peptide of the general formula

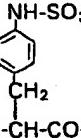
R₁-A-Tyr-C-D-Trp-Met-Y (2)

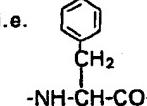
[R₁, A, C, D, and Y are the same as defined in the formula (1)] which is protected if necessary by masking its active group such as amino group with a suitable protective group is synthesized; and sulfate-esterified to convert the Tyr residue into Tyr(SO₃H).

For the peptide preparation specially requiring the elimination of a protective group after sulfate-esterification, the process disclosed in USP No. 4,330,466 is best suited in respect to the stabilization of the sulfate ester and the prevention of side reaction. This process comprises concentrating the sulfate-esterification reaction mixture and adding a solvent such as methanol, butanol, ethanol, dimethylformamide, or water and an aqueous solution of water-soluble salt of a divalent metal such as calcium or zinc to the concentrate to stabilize it, followed by eliminating the protective group.

The way of the sulfate-esterification is already known. For instance, the esterification is carried out by dissolving a peptide of the formula (2) in an inert solvent such as dimethylformamide or pyridine and adding a pyridine-anhydrous sulfuric acid complex in an amount of about 10 times the amount of the peptide. The reaction is preferably conducted first at a low temperature and then at room temperature, for 15-20 hours.

A peptide of the formula (1) wherein B is Phe-



(NHSO₃H), i.e., 

, is favorably prepared

in the following way:

A p-aminophenylalanine derivative of which the α-amino group is protected, e.g. Boc-Phe(NH₂)-OH, is reacted with a sulfonating reagent, e.g. a pyridine-sulfuric anhydride complex to sulfonate the p-amino group, the Boc-Phe(NHSO₃⁻)-OH is converted into a divalent metal salt by reacting with a salt of

divalent metal such as calcium in the same manner as mentioned above, the salt of Boc-Phe(NHSO₃⁻)-OH is condensed with a peptide fragment of the general formula



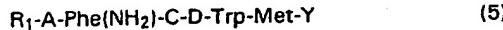
[C, D, and Y are the same as defined in the formula (1)] by a suitable method, e.g. the active ester process, DCC process, or the like, the resulting metal salt of the peptide of the general formula



[C, D, and Y are the same as defined in the formula (1)] is treated with trifluoroacetic acid or with some other reagent to eliminate the protective group, and the resulting peptide is further condensed with the necessary acyl group and amino acid (corresponding to R₁ and A).

A peptide of the formula (1) wherein B is Phe(NHSO₃H) is also prepared in the following way:

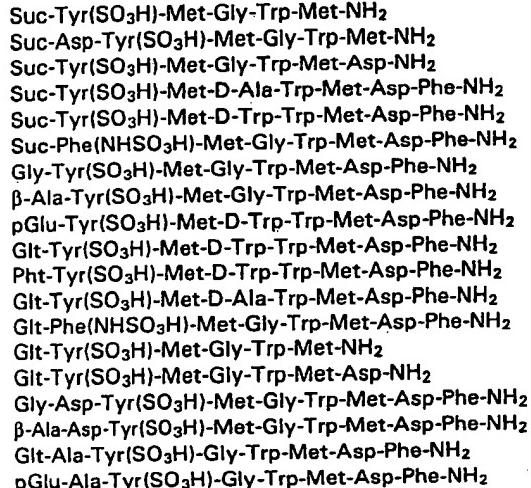
A peptide represented by the general formula



[R₁, A, C, D, and Y are the same as defined in the formula (1)] which is protected if necessary by masking its active group with a suitable protective group is synthesized and sulfonated to convert the Phe(NH₂) residue into Phe(NHSO₃H).

The thus obtained peptide of the invention, represented by the formula (1), can be desaltsed and purified according to common methods, for example, ion-exchange chromatography employing DEAE-Cellulose or the like, partition chromatography employing Sephadex LH-20 or Sephadex G-25, normal phase chromatography employing silica gel or the like, reversed phase chromatography employing ODS-silica gel or the like, and high performance liquid chromatography (HPLC).

Typical examples of the peptide represented by the formula (1) are as follows:



The peptide of the formula (1) can be made up into pharmaceutically acceptable salts, e.g. salts of

alkali metals such as sodium and potassium, salts of alkaline earth metals such as calcium, and salts of triethylamine and ammonium.

The peptide of the invention has physiological actions similar to that of «CCK-8» of the following formula



It has been found that peptides of the present invention have properties which are not found in CCK-8, in respect to the contraction of ginea pig gallbladder, enhancement of rat pancreatic juice secretion, and stimulation of pancreatic protein secretion, among the previously mentioned physiological actions of CCK-8.

A series of peptides of the invention having D-Ala or D-Trp as D of the formula (1) exhibits almost no action on the gallbladder while having markedly strong action on the pancreas. Moreover, a series of peptides of the invention having X-R₂-CO- as R₁ wherein X is amino is lower in the activity on the pancreas though equal or higher in the activity on the gallbladder, than CCK-8. Certain peptides of the invention are 2-6 times and 50-260 times as effective as tetragastrin in the acceleration of gastric secretion and the pancreatic external secretion, respectively.

Accordingly, certain peptides of the formula (1) according to the present invention are useful as medicines for a specific organ, (for example, the accelerator for the pancreatic function) and as specific reagents for laboratory tests (for example, the pancreatic function testing reagent and a contrast medium for gallbladder) and expected also to be gastrin-like peptides. Furthermore, other peptides of the invention can be expected antagonists of CCK-8 or gastrin.

The process for producing peptides of the invention is illustrated referring to the following examples; however, the invention is not limited to these examples. Abbreviations in the examples are as defined already.

Example 1

Preparation of Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂ (Compound I)

(1) Synthesis of Boc-Trp-Met-NH₂

5.78 g (0.039 mole) of H-Met-NH₂ was dissolved in 50 ml of DMF, then 5.46 ml of triethylamine and further 18.70 g (0.047 mole) of Boc-Trp-OSu were added under cooling with ice, and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and extracted with ethyl acetate. The extract was washed with 1N citric acid, saturated saline solution, saturated sodium bicarbonate solution, and saturated saline solution in series. The resulting organic layer was dried over anhydrous sodium sulfate, distilled in vacuo to remove the solvent, and solidified with hexane. The solid was recrystallized from ethyl acetate-hexane, giving 12.45 g of Boc-Trp-Met-NH₂; yield 73.5%, m.p. 155-157°C.

[α]_D²⁴ = -3.6° (C = 1, methanol).

Anal. Calcd. (%) for $C_{21}H_{30}N_4O_4S$: C, 50.04; H, 6.96; N, 12.86. Found (%): C, 50.14; H, 7.18; N, 12.68.

(2) Synthesis of Boc-Met-Gly-Trp-Met-NH₂

11.73 g (0.027 mole) of the dipeptide obtained in the above (1) was dissolved by adding 20 ml of trifluoroacetic acid containing 0.4 ml of ethanethiol and reacted at room temperature for 30 minutes. Then, a precipitate was formed by adding 200 ml of anhydrous ether to the reaction mixture and was filtered and dried. On the other hand, 12.98 g of Boc-Met-Gly-NHNH₂ was dissolved in 100 ml of DMF, and after cooling with dry ice-ethanol to —20°C or lower, was converted into azide by adding 20.25 ml of a 6N HC₆-dioxane mixture and 5.44 ml of isoamyl nitrite. The reaction mixture was neutralized by further adding 17.01 ml of triethylamine, and admixed with a solution of the above deprotected dipeptide in 100 ml of DMF. The mixture was stirred at —20°C for 2 hours and at 4°C for 17 hours. The resulting reaction mixture was extracted with ethyl acetate similarly to the above (1), the solvent was distilled off from the extract, and the residue was solidified with ether. The solid was filtered off, dried, and recrystallized from methanol-ether, giving 12.60 g of Boc-Met-Gly-Trp-Met-NH₂; yield 74.9%, m.p. 200–202°C.

$$[\alpha]_D^{24} = -24.3^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for $C_{28}H_{42}N_6O_6S_2$: C, 54.00; H, 6.80; N, 13.49. Found (%): C, 54.21; H, 6.81; N, 13.31.

(3) Synthesis of Boc-Tyr-Met-Gly-Trp-Met-NH₂

The protective group was eliminated from 6.85 g (0.011 mole) of the tetrapeptide obtained in the above (2), by using trifluoroacetic acid in the same manner as in above (2). The resulting tetrapeptide was dissolved in 50 ml of DMF, and 1.54 ml of triethylamine and 8.32 g (0.022 mole) of Boc-Tyr-Osu were added under cooling with ice. After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo and extracted with ethyl acetate. The extract was concentrated, and ether was added to deposit a solid, which was then filtered, dried, and recrystallized from methanol-ethyl acetate, giving 5.46 g of Boc-Tyr-Met-Gly-Trp-Met-NH₂; yield 63.1%, m.p. 195–198°C.

$$[\alpha]_D^{24} = -21.8^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for $C_{39}H_{51}N_7O_8S_2$: C, 56.54; H, 6.54; N, 12.47. Found (%): C, 56.67; H, 6.63; N, 12.25.

(4) Synthesis of Suc-Tyr-Met-Gly-Trp-Met-NH₂

The protective group was eliminated from 3.45 g (0.0044 mole) of the pentapeptide obtained in the above (3), by the same treatment as applied in the above (2). The treated pentapeptide was dissolved in DMF, and 1.23 ml of triethylamine and 0.88 g of succinic anhydride were added under cooling with ice. The mixture was stirred at room temperature for 1 day. The solvent was distilled off, 1N citric acid was added to the residue, and the resulting precipitate was filtered off, washed with water, and

recrystallized from methanol-ethyl acetate, giving 2.52 g of Suc-Tyr-Met-Gly-Trp-Met-NH₂; yield 72.8%, m.p. 125–127°C.

$$[\alpha]_D^{24} = -28.5^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for $C_{36}H_{47}N_7O_9S_2$: C, 55.02; H, 6.03; N, 12.48. Found (%): C, 54.91; H, 6.20; N, 12.42.

(5) Synthesis of Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂

1.57 g (0.002 mole) of the acylated peptide obtained in the above (4) was dissolved in a mixture of 20 ml of DMF and 2 ml of pyridine, and 3.18 g (0.020 mole) of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and at room temperature for 20 hours. The reaction mixture was concentrated in vacuo, 50 ml of 0.05 M aqueous ammonium carbonate solution was added, and the pH was adjusted to 8.5 by addition of aqueous ammonia. The solution was purified by chromatography on a DEAE-Cellulose column (5 × 15 cm), wherein the adsorption and washing were carried out by using 1.5 l of an 0.05 M buffer solution of ammonium carbonate-ammonium bicarbonate (pH 8.5) and eluted with 2.0 l of the same but 0.2 M buffer solution (pH 8.5). Eluted fractions were measured for ultraviolet absorbance in a wavelength of 278 nm, and thereby the fractions containing the intended product were collected, concentrated, and freeze-dried, giving 0.85 g of Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂ (Compound 1); yield 50.0%.

$$[\alpha]_D^{25} = -15.8^\circ \text{ (C = 0.5, 1N ammonia).}$$

Anal. Calcd. (%) for $C_{36}H_{47}N_7O_{12}S_3 \cdot NH_3 \cdot H_2O$: C, 47.99; H, 5.82; N, 12.44. Found (%): C, 47.94; H, 5.59; N, 12.00.

Amino acid analysis by acidolysis:

Gly 1.00 (1), Met 1.98 (2), Tyr 1.01 (1)

Infrared absorption spectroscopy of the product indicates a strong absorption peak characteristic of sulfate ester at 1050 cm⁻¹.

Example 2

Preparation of Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-NH₂ (Compound II)

(1) Synthesis of Boc-Met-Asp-NH₂

12.47 g (0.035 mole) of Z-Asp(OBz)-NH₂ (m.p. 118–120°C. $[\alpha]_D^{24} = -2.6^\circ$ (C = 1, methanol)). Anal. Calcd. (%) for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found (%): C, 64.04; H, 5.73; N, 7.73 was dissolved in a mixture of 300 ml of methanol and 35 ml of 1N HC₆ and subjected to contact reduction with hydrogen (at room temperature for 6 hours) in the presence of 1.75 g of a 10% Pd-carbon catalyst. The catalyst was then filtered off, the solvent was distilled off, and the residue was dried in vacuo. On the other hand, 9.36 g (0.035 mole) of Boc-Met-OH was dissolved in 50 ml of THF, the solution was cooled to —20°C, and 3.85 ml of N-methylmorpholine and 4.62 ml of isobutyl chloroformate were added. The resulting mixed anhydride was admixed with a solution of the above obtained H-Asp-NH₂ in 50 ml of DMF (containing 4.90 ml of triethylamine). The mixture was stirred at 0°C for

methanol-ether giving 19.90 g of Boc-Met-D-Ala-Trp-Met-Asp-Phe-NH₂; yield 69.2%, m.p. 199-202°C.

$[\alpha]_D^{24} = -30.0^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₄₂H₅₈N₈O₁₀S₂: C, 56.11; H, 6.50; N, 12.46. Found (%): C, 56.00; H, 6.93; N, 12.46.

(3) Synthesis of Boc-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH₂

In the same manner as in (3) of Example 1, 9.90 g (0.011 mole) of the hexapeptide obtained in the preceding (2) was condensed with 8.23 g of Boc-Tyr-OSu. The product was recrystallized from methanol-ethyl acetate, giving 8.36 g of Boc-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH₂; yield 71.5%, m.p. 202-205°C.

$[\alpha]_D^{24} = -25.8^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₅₁H₆₈N₉O₁₃S₂·H₂O: C, 56.70; H, 6.44; N, 11.67. Found (%): C, 57.00; H, 6.54; N, 11.22.

(4) Synthesis of Suc-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH₂

In the same manner as in (4) of Example 1, the protective group was eliminated from 4.02 g (0.0038 mole) of the heptapeptide obtained in the preceding (3). The deprotected heptapeptide was reacted with 0.76 g (0.0076 mole) of succinic anhydride. The product was recrystallized from methanol-ethyl acetate, giving 3.85 g of Suc-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH₂; yield 95.8%, m.p. 200-203°C.

$[\alpha]_D^{24} = -29.3^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₅₀H₆₃N₉O₁₃S₂: C, 56.54; H, 5.98; N, 11.87. Found (%): C, 56.26; H, 6.36; N, 12.05.

(5) Synthesis of Suc-Tyr(SO₃H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂

In the same manner as in (6) of Example 2, 2.12 g (0.002 mole) of the Suc-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH₂ obtained in the preceding (4) was sulfated and purified by chromatography on a DEAE-Cellulose column (5 × 15 cm). Thus, 1.25 g of Suc-Tyr(SO₃H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂ (Compound III) was obtained as a lyophilized product; yield 54.9%.

Anal. Calcd. (%) for C₅₀H₆₃N₉O₁₀S₂·NH₃·3H₂O: C, 49.50; H, 5.98; N, 11.54. Found (%): C, 49.67; H, 5.71; N, 11.52.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Gly 0.99 (1), Met 2.00 (2), Tyr 1.01 (1), Phe 1.01 (1)

Infrared absorption spectrum: 1050 cm⁻¹ (sulfate ester).

Example 4
Preparation of Suc-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ (Compound IV)

(1) Synthesis of Boc-D-Trp-Trp-Met-Asp-Phe-NH₂

In the same manner as in (1) of Example 1, 10.45 g (0.015 mole) of Boc-Trp-Met-Asp-Phe-NH₂ (cf. (1)

of Example 3) was condensed with 9.00 g (0.022 mole) of Boc-Trp-OSu. The resulting reaction liquid was concentrated, and a precipitate was formed by adding 1N citric acid and washed with water. Recrystallization thereof from methanol-water gave 8.09 g of Boc-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 61.1%, m.p. 225-227°C.

$[\alpha]_D^{24} = -24.0^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₄₅H₆₄N₈O₉S: C, 61.21; H, 6.16; N, 12.69. Found (%): C, 61.44; H, 6.30; N, 12.55.

(2) Synthesis of Boc-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

In the same manner as in (2) of Example 3, 7.94 g (0.009 mole) of the pentapeptide obtained in the preceding (1) was condensed with 3.37 g of Boc-Met-OH. The product was recrystallized from methanol-water, giving 7.00 g of Boc-Met-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 76.7%, m.p. 195-197°C.

$[\alpha]_D^{24} = -28.1^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₅₀H₆₃N₉O₁₀S₂: C, 59.21; H, 6.26; N, 12.43. Found (%): C, 59.08; H, 6.37; N, 12.24.

(3) Synthesis of Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

In the same manner as in (3) of Example 1, 3.55 g (0.0035 mole) of the hexapeptide obtained in the preceding (2) was freed of the protective group and then condensed with 2.65 g of Boc-Tyr-OSu. The product was recrystallized from methanol-water, giving 3.51 g of Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 85.2%, m.p. 204-206°C.

$[\alpha]_D^{24} = -26.3^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₅₉H₉₂N₁₀O₁₂S₂: C, 60.19; H, 6.16; N, 11.90. Found (%): C, 59.93; H, 6.28; N, 11.35.

(4) Synthesis of Suc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

In the same manner as in (4) of Example 1, 3.41 g (0.0029 mole) of the heptapeptide obtained in the preceding (3) was deprotected, and then reacted with 0.58 g of succinic anhydride. The product was recrystallized from methanol-ether, giving 3.17 g of Suc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 92.8%, m.p. 198-201°C.

$[\alpha]_D^{24} = -31.1^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₆₈H₈₈N₁₀O₁₃S₂: C, 59.17; H, 5.82; N, 11.90. Found (%): C, 59.40; H, 6.06; N, 11.75.

(5) Synthesis of Suc-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

In the same manner as in (5) of Example 1, 2.36 g (0.002 mole) of the acylated peptide obtained in the preceding (4) was sulfated with 3.18 g of a pyridine-sulfuric anhydride complex. The product was purified by chromatography on a DEAE-Cellulose column (5 × 15 cm), wherein 2 l of a 30% methanol-0.5 M ammonium carbonate buffer solution (pH 8.5) was used for elution. Thus, 1.28 g of Suc-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

(Compound IV) was obtained as a lyophilized product; yield 50.9%.

$$[\alpha]_D^{24} = -15.0^\circ \text{ (C = 1, 1N ammonia).}$$

Anal. Calcd. (%) for $C_{58}H_{68}N_{10}O_{16}S_3 \cdot NH_3 \cdot 3H_2O$: C, 52.44; H, 5.84; N, 11.60. Found (%): C, 52.21; H, 5.71; N, 11.91.

Amino acid analysis by acidolysis:

Asp 1.03 (1), Met 1.98 (2), Tyr 1.00 (1), Phe 0.99 (1).

Example 5

Preparation of Glt-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ (Compound V)

Similarly to (4) of Example 4, 1.77 g (0.0015 mole) of the Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ obtained in (3) of Example 4 was deprotected and reacted with 0.342 g of glutaric anhydride. The product was recrystallized from methanol-ether, giving 1.50 g of Glt-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 83.9%, m.p. 208-210°C.

$$[\alpha]_D^{24} = -25.7^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for $C_{59}H_{70}N_{10}O_{13}S_2 \cdot H_2O$: C, 58.60; H, 6.00; N, 11.58. Found (%): C, 58.49; H, 6.08; N, 11.52.

Similarly to (5) of Example 4, 1.30 g of the above peptide was sulfated and purified by chromatography on a DEAE-Cellulose column (4 × 15 cm). Thus, 683 mg of Glt-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ (Compound V) was obtained as a lyophilized product; yield 49.3%.

Anal. Calcd. (%) for $C_{59}H_{70}N_{10}O_{16}S_3 \cdot NH_3 \cdot 4H_2O$: C, 52.09; H, 6.00; N, 11.32. Found (%): C, 51.92; H, 6.11; N, 11.33.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Met 1.94 (2), Tyr 1.03 (1), Phe 0.98 (1).

Example 6

Preparation of Pht-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂

Similarly to (4) of Example 4, 1.77 g (0.0015 mole) of the Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ obtained in (3) of Example 4 was deprotected and condensed with 0.444 g of phthalic anhydride. The resulting product was recrystallized from methanol-ether, giving 1.79 g of Pht-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-NH₂; yield 97.4%, m.p. 180-183°C.

$$[\alpha]_D^{24} = -31.4^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for $C_{62}H_{68}N_{10}O_{13}S_2 \cdot H_2O$: C, 59.89; H, 5.67; N, 11.26. Found (%): C, 59.55; H, 5.97; N, 11.45.

Similarly to (5) of Example 4, 1.59 g of the above peptide was sulfated and purified by chromatography on a DEAE-Cellulose column (4 × 15 cm). Thus, 638 mg of Pht-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂ (Compound VI) was obtained as a lyophilized product; yield 37.6%.

$$[\alpha]_D^{24} = -18.3^\circ \text{ (C = 1, 1N ammonia).}$$

Anal. Calcd. (%) for $C_{62}H_{68}N_{10}O_{16}S_3 \cdot NH_3 \cdot 4H_2O$: C, 53.40; H, 5.71; N, 11.05. Found (%): C, 53.61; H, 5.65; N, 10.59.

Amino acid analysis by acidolysis:

Asp 1.06 (1), Met 1.98 (2), Tyr 0.96 (1), Phe 0.99 (1).

Example 7

Preparation of Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound VII)

(1) Synthesis of Boc-Phe(NH₂)-OH

3.27 g (0.014 mole) of H-Phe(NO₂)-OH and 3.26 mL of triethylamine were dissolved in 50 mL of water, and 50 mL of a dioxane solution of 4.10 g (0.017 mole) of a Boc-S reagent, e.g. t-butyl S-4,6-dimethyl pyrimidin-2-yl thiocarbonate (made by Kokusan Kagaku Co., Ltd.) was added under cooling with ice. After one day stirring at room temperature, the reaction mixture was diluted with 50 mL of water and washed with ethyl acetate. The aqueous layer, cooled with ice, was acidified to pH 2 by adding 6N HCl. The mixture was extracted with ethyl acetate, and the extract was washed with 1N HCl and then saturated saline solution, and dried over anhydrous sodium sulfate. The ethyl acetate was distilled off in vacuo, hexane was added to the residue, and the solid deposit was filtered and dried. Recrystallization thereof from ethyl acetate-hexane gave 4.04 g of Boc-Phe(NO₂)-OH; yield 84.0%, m.p. 110-112°C.

$$[\alpha]_D^{24} = +7.7^\circ \text{ (C = 1, methanol).}$$

Anal. Calcd. (%) for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85; N, 9.03. Found (%): C, 54.13; H, 5.77; N, 9.10.

3.93 g (0.013 mole) of the above obtained Boc-Phe(NO₂)-OH was dissolved in 50 mL of methanol and hydrogenated over 1.3 g of a 5% Pd-carbon catalyst at room temperature for 8 hours. Then, the catalyst was filtered off, and the filtrate was concentrated in vacuo. Hexane was added to the residual to form a precipitate, which was then recrystallized from ethyl acetate-ether, giving 2.39 g of Boc-Phe(NH₂)-OH; yield 67.1%, m.p. 126-128°C.

$$[\alpha]_D^{24} = +26.6^\circ \text{ (C = 1, methanol).}$$

Anal. Calcd. (%) for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found (%): C, 59.79; H, 7.15; N, 9.85.

(2) Synthesis of Boc-Phe($NHSO_3^-$)-O⁻-calcium salt

1.26 g (0.0045 mole) of the Boc-Phe(NH₂)-OH obtained in the preceding (1) was dissolved in a mixture of 20 mL of DMF and 2 mL of pyridine, and 3.58 g of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and then at room temperature for 20 hours. The resulting reaction mixture was concentrated in vacuo, 30 mL of ice-cold water was added to the residue to dissolve it, and 27 mL of 1 M aqueous calcium acetate solution was added immediately. The resulting calcium sulfate precipitate was removed by centrifugation, carbon dioxide gas was blown into the supernatant, and the resulting calcium carbonate precipitate was removed by centrifugation. The resulting supernatant was concentrated in vacuo. The residue was

recrystallized from ethanol-ether, giving Boc-Phe-(NHSO₃⁻)-O⁻·Ca²⁺.

(3) Synthesis of Boc-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

1.71 g (0.0045 mole) of the sulfonated amino acid obtained in the preceding (2) was dissolved in 20 ml of DMF, then 0.57 g of N-hydroxysuccinimide and 0.93 g of dicyclohexylcarbodiimide were added, and the mixture was stirred overnight at 4°C. The precipitated urea compound was filtered off, the filtrate was concentrated in vacuo, ether was added to the residue, and the resulting precipitate was filtered and dried, giving Boc-Phe(NHSO₃⁻)-OSu·1/2 Ca salt.

On the other hand, 2.66 g (0.003 mole) of Boc-Met-Gly-Trp-Met-Asp-Phe-NH₂ [m.p. 195-197°C. [α]_D²⁴ = -30.0° (C = 1, DMF). Anal. Calcd. (%) for C₄₁H₅₆N₈O₁₂S₂: C, 55.64; H, 6.38; N, 12.66. Found (%): C, 55.85; H, 6.55; N, 12.54. cf. M.A. Ondett et al., Journal of the American Chemical Society, 92, 195 (1970)] was dissolved in 6 ml of trifluoroacetic acid containing 0.2 ml of ethandithiol, and the solution was allowed to stand at room temperature for 30 minutes. Then, 70 ml of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. The deprotected peptide was dissolved in DMF, and 0.42 ml of triethylamine was added to the solution cooled with ice.

To the resulting solution was added to the above obtained active ester [Boc-Phe(NHSO₃⁻)-OSu·1/2 Ca salt], and the mixture was stirred overnight at room temperature. The reaction mixture was then concentrated in vacuo, and a precipitate was formed by adding ethyl acetate to the residue. The precipitate was filtered and dried, giving 2.20 g of Boc-Phe-(NHSO₃⁻)-Met-Gly-Trp-Met-Asp-Phe-NH₂·Ca salt.

(4) Synthesis of Suc-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

The heptapeptide calcium salt obtained in the preceding (3) was dissolved in 10 ml of trifluoroacetic acid containing 0.2 ml of ethandithiol, and the solution was allowed to stand at 0°C for 30 minutes. To the reaction mixture was added 100 ml of anhydrous ether, and the resulting precipitate was filtered and dried. This deprotected peptide was dissolved in 30 ml of DMF, and reacted with 1.3 g of succinic anhydride in the same manner as in (4) of Example 1. The resulting reaction mixture was concentrated in vacuo, and purified in the same manner as in (5) of Example 1 by chromatography on a DEAE-Cellulose column (5 × 15 cm). Thus, 460 mg of Suc-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound VII) was obtained as a lyophilized product.

[α]_D²⁵ = -17.0° (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for C₄₉H₆₂N₁₀O₁₅S₂·3H₂O: C, 49.82; H, 5.80; N, 11.86. Found (%): C, 49.63; H, 5.61; N, 11.96.

Amino acid analysis by acidolysis:

Asp 1.08 (1), Gly 1.05 (1), Met 2.04 (2), Phe 1.00 (1), Phe(NH₂) 0.84 (1).

Example 8

Preparation of Gly-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound VIII)

(1) Synthesis of Boc-Gly-Asp-Tyr-NHNH₂

4.81 g (0.009 mole) of Z-Asp(OBz)-Tyr-OMe [m.p. 126-127°C. [α]_D²⁴ = -4.8° (C = 1, methanol). Anal. Calcd. (%) for C₂₉H₃₀N₂O₈: C, 65.16; H, 5.66; N, 5.24. Found (%): C, 65.14; H, 5.71; N, 5.18] was dissolved in a mixture of 150 ml of methanol and 9 ml of 1N HCl and hydrogenated over 0.9 g of a 10% Pd-carbon catalyst at room temperature for 6 hours. Then the catalyst was filtered off, and the filtrate was distilled in vacuo to dryness. The resulting dipeptide was condensed with a mixed anhydride which had been prepared from 1.89 g of Boc-Gly-OH in the same manner as in (1) of Example 2. The product was recrystallized from ethyl acetate-ether, giving 3.09 g of Boc-Gly-Asp-Tyr-OMe; yield 71.8%, m.p. 97-99°C.

[α]_D²⁴ = -14.1° (C = 1, methanol).

Anal. Calcd. (%) for C₂₁H₂₉N₃O₉: C, 53.96; H, 6.25; N, 8.99. Found (%): C, 53.95; H, 6.68; N, 8.69.

1.83 g of the above obtained tripeptide methyl ester was dissolved in 15 ml of methanol, then 1.08 ml of 90% hydrazine hydrate was added to the solution, and the mixture was stirred at room temperature for 17 hours. Ether was added to the reaction mixture in which a precipitate had formed. The precipitate was filtered, dried, and washed again with 5% acetic acid, giving 1.68 g of Boc-Gly-Asp-Tyr-NHNH₂; yield 92.1%, m.p. 149-151°C.

[α]_D²⁵ = -23.6° [C = 0.5, DMF - acetic acid (1:1)].

Anal. Calcd. (%) for C₂₀H₂₉N₅O₈: C, 51.39; H, 6.25; N, 14.98. Found (%): C, 51.14; H, 6.37; N, 15.09.

(2) Synthesis of Boc-Gly-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂

0.885 g (0.001 mole) of Boc-Met-Gly-Trp-Met-Asp-Phe-NH₂ [cf. (3) of Example 7] was similarly treated with 6 ml of trifluoroacetic acid containing 0.2 ml of ethandithiol and condensed with 0.701 g of the tripeptide obtained in the preceding (1), in the same manner as in (2) of Example 1 according to the azide process. The product was precipitated by adding 1N citric acid, washed with water, and recrystallized from methanol, giving 0.97 g of Boc-Gly-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂; yield 79.5%, m.p. 197-200°C.

[α]_D²⁴ = -39.5° (C = 1, DMF).

Anal. Calcd. (%) for C₅₆H₇₃N₁₁O₁₆S₂·H₂O: C, 54.31; H, 6.10; N, 12.44. Found (%): C, 54.13; H, 6.06; N, 12.18.

(3) Synthesis of Gly-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

0.488 g (0.0004 mole) of the nonapeptide obtained in the preceding (2) was dissolved in a mixture of 10 ml of DMF and 1 ml of pyridine. 0.637 g of a pyridine-sulfuric anhydride complex was added under cooling with ice, and the mixture was stirred at 0°C for 30 minutes and then at room temperature

for 17 hours. The resulting reaction mixture was concentrated in vacuo, and 20 ml of methanol, 20 ml of butanol, and 12 ml of 0.5 M aqueous calcium acetate solution were added to the residue. The resulting precipitate of calcium sulfate was removed by centrifugation; and the supernatant was distilled in vacuo to dryness. The residue was washed with water, and 0.503 g of Boc-Gly-Asp-Tyr(SO₃⁻)-Met-Gly-Trp-Met-Asp-Phe-NH₂·Ca salt was obtained.

This sulfated peptide·Ca salt was treated at 0°C for 30 minutes with 5 ml of trifluoroacetic acid containing 0.1 ml of ethandithiol. The precipitate formed by adding then anhydrous ether to the reaction mixture was filtered and purified by chromatography on a DEAE-Cellulose column (3 × 7 cm), wherein 1 l of an 0.3 M ammonium carbonate buffer solution (pH 8.5) was used for elution. Thus, 0.243 g of Gly-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound VIII) was obtained as a lyophilized product; yield 50.8%.

$[\alpha]_D^{25} = -26.2^\circ$ (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for C₅₁H₈₅N₁₁O₁₇S₃·NH₃·5H₂O: C, 46.85; H, 6.01; N, 12.86. Found (%): C, 46.80; H, 5.86; N, 12.81.

Amino acid analysis by acidolysis:

Asp 2.05 (2), Gly 1.99 (2), Met 2.00 (2), Tyr 0.97 (1), Phe 0.99 (1).

Infrared absorption spectrum: 1050 cm⁻¹ (sulfate ester).

Example 9

Preparation of β-Ala-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound IX)

(1) Synthesis of Boc-β-Ala-Asp-Tyr-NHNH₂

In the same manner as in (1) of Example 8, 3.25 g of Boc-β-Ala-Asp-Tyr-OMe was obtained from 4.81 g (0.009 mole) of Z-Asp(OBz)-Tyr-OMe and 2.04 g (0.011 mole) of Boc-β-Ala-OH; yield 75.0%, m.p. 88-90°C.

$[\alpha]_D^{24} = -22.4^\circ$ (C = 1, methanol).

Anal. Calcd. (%) for C₂₂H₃₁N₃O₉: C, 54.88; H, 6.48; N, 8.73. Found (%): C, 54.97; H, 6.64; N, 8.42.

Also in the same manner as in (1) of Example 8, 1.56 g of the above tripeptide methyl ester was converted into a hydrazide derivative, giving 1.18 g of Boc-β-Ala-Asp-Tyr-NHNH₂; yield 75.7%, m.p. 183-186°C.

$[\alpha]_D^{25} = -38.4^\circ$ [C = 0.5, DMF - acetic acid (1:1)].

Anal. Calcd. (%) for C₂₁H₃₁N₅O₈: C, 52.38; H, 6.49; N, 14.55. Found (%): C, 52.12; H, 6.47; N, 14.45.

(2) Synthesis of Boc-β-Ala-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂

In the same manner as in (2) of Example 8, 0.977 g of Boc-β-Ala-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂ was obtained according to the azide method by condensation of 0.885 g of Boc-Met-Gly-Trp-Met-Asp-Phe-NH₂ with 0.722 g of Boc-β-Ala-Asp-Tyr-NHNH₂ obtained in the preceding (1); yield 79.1%, m.p. 207-209°C.

$[\alpha]_D^{24} = -41.0^\circ$ (C = 1, DMF).

Anal. Calcd. (%) for C₅₇H₇₅N₁₁O₁₆S₂: C, 55.46; H, 6.12; N, 12.48. Found (%): C, 55.65; H, 6.19; N, 12.32.

(3) Synthesis of β-Ala-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

In the same manner as in (3) of Example 8, sulfation, calcium salt formation, deprotection and purification were conducted on 0.494 g (0.4 mmole) of the nonapeptide obtained in the preceding (2), giving 0.243 g of β-Ala-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂ (Compound IX); yield 50.1%.

$[\alpha]_D^{24} = -33.2^\circ$ (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for C₅₂H₆₇N₁₁O₁₇S₃·NH₃·4H₂O: C, 47.92; H, 6.03; N, 12.89. Found (%): C, 48.05; H, 5.64; N, 12.66.

Amino acid analysis by acidolysis:

Asp 2.09 (2), Gly 0.98 (1), Met 2.07 (2), Tyr 1.05 (1), Phe 0.84 (1), β-Ala 0.97 (1).

Infrared absorption spectrum: 1050 cm⁻¹ (sulfate ester).

Pharmacological test I

(1) Gallbladder contracting activity

This test was in accordance with the method of G. Bertaccini et al. [Br. J. Pharmacol., 34, 291-310 (1968)].

Male Hartley guinea pigs (weighing 400-600 g) were anesthetized with urethane and fixed at the supine position. After laparotomy, the top of the gallbladder was clamped with a Serre-fine which was connected to a FD pickup (model: SB-IT, NIHON KOHDEN KOGYO Co., Ltd.), and the contraction was recorded on a recorder (model: PJB-3012, made by the above company) through a preamplifier (model: RUP-25, made by the above company). A standard solution and the invented peptide solutions were injected into the jugular vein, and the relative potency was determined from the peak height of the gallbladder contraction. Results of the test are shown in Table 1.

Table 1 - Gallbladder contracting activity

| | Peptide | Relative potency |
|------------|---------|------------------|
| Compound I | | 0 |
| » II | | 0 |
| » III | | 8.7 |
| » IV | | 5.0 |
| » V | | < 1.0 |
| » VI | | < 1.0 |
| » VII | | < 1.0 |
| » VIII | | 97 |
| » IX | | 113 |
| CCK - 8 | | 100 |

As is shown in Table 1, Compounds VIII and IX exhibited comparable activities with CCK-8 and activities of Compounds III and IV were mild; while Compounds V, VI, and VII exhibited little activity and Compounds I and II no activity.

(2) Accelerating on pancreatic external secretion

This test was conducted in accordance with the method of Dockray [J. Physiol., 225, 679-692 (1972)]. Male Wistar rats (weighing about 280 g) were deprived for 24 hours and under anesthesia the gastric pylorus and the duodenal side end of the common hepatic duct were ligated. A canula for collection of pancreatic juice was inserted into the duct in the retrograde fashion. The bile was led into the duodenum. Varying concentrations of the control solution (CCK-8 solution) and solutions of the invented peptides in a volume of 50 μ l were administered intravenously every one hour, thus determining the increment in the secretion rate of the pancreatic juice and protein during 30 minutes after administration, wherein the amount of the whole protein secreted was determined from the ultraviolet absorbance at 280 nm of the pancreatic juice secreted. Results thereof are shown in Figs. 1-4.

Referring to the results of the increased amount of the pancreatic juice secreted, the activities of Compounds IV, V, VI, and VIII were comparable to that of CCK-8 and the activity of Compound IX was about half of that of CCK-8, while Compounds I, II, and VII exhibited no active effect.

(3) The use of the Compounds of IV, V, VI, and VII, and the salts of the compounds for accelerating the pancreatic function will be described below

Firstly, these compounds and salts are mixed with well-known pharmaceutical carriers to prepare pharmaceutical compositions. The carriers include diluents and excipients, e.g., filler, extender, binder, wetting agent, disintegrator, and surfactant, etc. The carriers are selected depending upon a dosage form.

The Compounds of IV, V, VI, and VII, and the salts of the compounds can be contained in the pharmaceutical composition in various amount without restriction. Generally, it is preferable that these compounds and salts are contained in an amount of 1-70 wt. % based on the total composition.

The thus prepared accelerator for pancreatic function is administrated by a method depending upon a dosage form. The administration method is not restricted particularly. For example, tablets, pills, solutions, suspensions, emulsions, granules, and capsules are suitable for oral administration; parenteral solutions which contain singly the active ingredient or a mixture of conventional cosolvents such as dextrose and amino acids with the active ingredient are suitable for intravenous injection; the active ingredient is, if necessary, singly administrated intramuscularly, intracutaneously, subcutaneously, or intraperitoneally; suppositories are suitable for rectal administration; and nasal drops are suitable for nasal administration. A suitable dosage range is selected depending upon a using object, the conditions of a patient, and the like. A pharmaceutical composition containing the Compound of IV, V, VI, or VII, or a salt of the compounds is administrated to a human being of

weight 60 kg for 1-4 times per day in an amount of about 4 μ g to 2 mg of the compound or the salt thereof per kg of body weight per day. With regard to acute toxicity, even when the compound or a salt of the compounds was subcutaneously injected to a mouse in a dose of 2 mg/kg, no toxicity was observed.

Example 10

Preparation of Glt-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂ (Compound X)(1) Synthesis of Glt-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂

1.00 g of Boc-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂ (m.p. 197-200°C. Anal. Calcd. (%)) for C₄₈H₆₁N₉O₁₂S: C, 58.35; H, 6.22; N, 12.75. Found (%): C, 58.35; H, 6.43; N, 12.61) was dissolved in 5 ml of trifluoroacetic acid containing 0.2 ml of ethanedithiol and reacted at room temperature for 30 minutes. Then 100 ml of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. This deprotected heptapeptide was dissolved in 20 ml of DMF containing 0.14 ml of triethylamine, and 0.40 g of glutaric anhydride was added under cooling with ice. After 17-hour stirring at 4°C, the reaction mixture was distilled in vacuo to remove the solvent, and 1N citric acid was added to the residue to form a precipitate. It was washed with water and recrystallized from 20 ml of methanol, giving 0.77 g of Glt-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂; yield 76.1%, m.p. 167-170°C.

$$[\alpha]_D^{24} = -38.5^\circ \text{ (C = 1, DMF).}$$

Anal. Calcd. (%) for C₄₈H₅₉N₉O₁₃S: C, 57.53; H, 5.93; N, 12.58. Found (%): C, 57.42; H, 5.99; N, 12.22.

(2) Synthesis of Glt-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂

301 mg of the acylated peptide obtained in the preceding (1) was dissolved in a mixture of 10 ml of DMF and 1 ml of pyridine, and 477 mg of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and then at room temperature for 20 hours. The reaction mixture was concentrated in vacuo, 30 ml of 0.05 M aqueous ammonium carbonate solution was added to the residue, and the pH was adjusted to 8.5 with aqueous ammonia. The solution was purified by chromatography on a DEAE-Cellulose column (4 cm x 12 cm), wherein the adsorption and washing was carried out by using 500 ml of an 0.05 M buffer solution of ammonium carbonate-ammonium bicarbonate (pH 8.5) and the elution by using 700 ml of the same but 0.2 M buffer solution (pH 8.5). The active fractions eluted were collected, concentrated, and lyophilized, giving 163 mg of Glt-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂ (Compound X); yield 50.2%.

Anal. Calcd. (%) for C₄₈H₅₉N₉O₁₆S₂·NH₃·4H₂O: C, 49.22; H, 6.02; N, 11.96. Found (%): C, 49.22; H, 5.79; N, 12.12.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Gly 0.96 (1), Ala 1.02 (1), Met 0.94 (1), Tyr 1.00 (1), Phe 0.98 (1).

Infrared absorption spectrum: 1050 cm⁻¹ (sulfate ester).

Example 11

Preparation of pGlu-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂ (Compound XI)

(1) Synthesis of pGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂

1.51 g of Boc-Gly-Trp-Met-Asp-Phe-NH₂ was dissolved in 5 ml of trifluoroacetic acid containing 0.2 ml of ethandithiol, and reacted at room temperature for 30 minutes. Then 100 ml of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. This pentapeptide, dissolved in 20 ml of DMF containing 0.28 ml of triethylamine, was condensed with 0.76 g of pGlu-Ala-Tyr-NHNH₂ [m.p. 269-270°C]. Anal. Calcd. (%) for C₁₇H₂₃N₅O₆: C, 54.10; H, 6.14; N, 18.56. Found (%): C, 54.00; H, 6.28; N, 18.74] according to the azide method wherein, the hydrazide was reacted after being converted with isoamyl nitrite into the azide. Then the solvent was distilled off in vacuo, and 1N citric acid was added to the residual liquid to form a precipitate. It was washed with water and recrystallized from dimethylformamide-methanol, giving 1.68 g of pGlu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂; yield 84.1%, m.p. 207-209°C.

[α]_D²⁴ = -34.9° (C = 1, DMF).

Anal. Calcd. (%) for C₄₈H₅₈N₁₀O₁₂S: C, 57.70; H, 5.85; N, 14.02. Found (%): C, 57.60; H, 6.15; N, 14.15.

(2) Synthesis of pGlu-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂

1.00 g of the octapeptide obtained in the preceding (1) was dissolved in a mixture of 10 ml of DMF and 1 ml of pyridine, 1.60 g of a pyridine-sulfuric anhydride complex was added under cooling with ice, and the mixture was stirred at 0°C for 30 minutes and then at room temperature for 17 hours. The resulting reaction mixture was concentrated in vacuo, 50 ml of an 0.05 M aqueous ammonium carbonate solution was added to the residue, and the pH was adjusted to 8.5 by adding aqueous ammonia. The solution was purified by ion exchange chromatography on a DEAE-Cellulose column (4 cm x 12 cm). The active fractions eluted were collected, concentrated, and lyophilized, giving 563 mg of pGlu-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂ (Compound XI), yield 52.2%.

Anal. Calcd. (%) for C₄₈H₅₈N₁₀O₁₅S₂·NH₃·4H₂O: C, 49.35; H, 5.95; N, 13.19. Found (%): C, 49.51; H, 5.95; N, 13.02.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Glu 0.96 (1), Gly 1.04 (1), Ala 0.98 (1), Met 1.00 (1), Tyr 1.03 (1), Phe 0.94 (1).

Infrared absorption spectrum: 1050 cm⁻¹ (sulfate ester).

Pharmacological test II

(1) Gastric acid secretion accelerating activity

This test was conducted with Shay rats [H. Shay et al., Gastroenterology, 5, 43 (1945)] prepared. Employing male Wistar rats (weighing 270-310 g) deprived for 24 hours, the gastric pylorus was ligated under ether-anesthesia. Immediately thereafter, the invented peptides and tetragastrin as a control were administered intravenously. Gastric juice was withdrawn 2 hours after administration, and its amount and the total amount of acid secreted were measured to determine the relative potency based on tetragastrin. Results of the test are shown in Table 2.

Table 2 - Gastric acid secretion accelerating activity

| Peptide | Relative potency |
|--------------|------------------|
| Compound X | 6.1 |
| » XI | 2.0 |
| Tetragastrin | 1.0 |

(2) Pancreatic external secretion accelerating activity

The test was conducted in the same manner as stated already. The increment in the secretion rate of the protein in the pancreatic during 30 minutes after administration of the test solution was determined from the ultraviolet absorbance at 280 nm of the pancreatic juice. The found values were compared with that of tetragastrin. Results thereof are shown in Table 3.

Table 3 - Pancreatic external secretion accelerating activity

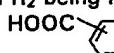
| Peptide | Relative potency |
|--------------|------------------|
| Compound X | 258.8 |
| » XI | 50.0 |
| Tetragastrin | 1.0 |

Claims

1. Novel peptides represented by the following formula



wherein R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or



Asp, Ala, or merely a chemical bond; B denotes Tyr(SO₃H) or Phe(NHSO₃H); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with

the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or HOOC-CO-, B is Tyr(SO₃H), C is Met, and D is Gly, and pharmacologically acceptable salts thereof.

2. Novel peptides and salts according to claim 1, wherein R₁ is NH₂-R₂-CO-, R₂ being lower alkylene of 1-6 carbon atoms.

3. Novel peptides and salts according to claim 1 or 2, wherein B is Phe(NHSO₃H).

4. Novel peptides and salts according to any preceding claim, wherein D is D-Ala or D-Trp.

5. Novel peptides and salts according to any preceding claim, wherein Y is NH₂ or Asp-NH₂.

6. The novel peptides and pharmacologically acceptable salts thereof according to any preceding claim, wherein R₁ is pGlu, X-R₂-CO-, X being carboxyl group or amino group and R₂ being lower alkylene group of 1-6 carbon atoms, or HOOC-CO-, A is Ala, and C is merely a chemical bond.

7. A novel peptide according to claim 1, selected from:

Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂,
Suc-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂,
Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-NH₂,
Suc-Tyr(SO₃H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,
Suc-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Suc-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Gly-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
β-Ala-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
pGlu-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Pht-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO₃H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,
Glt-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂,
Glt-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-NH₂,
Gly-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
β-Ala-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂, and
pGlu-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂.

8. A novel peptide according to claim 1 selected from:

Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-NH₂,
Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-NH₂,
Suc-Tyr(SO₃H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,
Suc-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Suc-Phe(NHSO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Pht-Tyr(SO₃H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Gly-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
β-Ala-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂, and
pGlu-Ala-Tyr(SO₃H)-Gly-Trp-Met-Asp-Phe-NH₂.

9. A pharmacologically acceptable peptide salt according to any preceding claim selected from alkali metal salts, alkali earth metal salts, organic amine salts, and ammonium salts.

10. A process for the preparation of novel peptides represented by the following formula

R₁-A-Tyr(SO₃H)-C-D-Trp-Met-Y

wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOC-CO-; A denotes

5 Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or

10 HOOC-CO-, C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that the Tyr-residue in the peptide of the following formula

R₁-A-Tyr-C-D-Trp-Met-Y

(2)

wherein; R₁, A, C, D, and Y are the same as defined above, or the peptide in which active groups such as amino groups are protected by suitable protecting groups, is subjected to the sulfate-ester reaction to convert the Tyr-residue to Tyr(SO₃H) residue.

11. A process for the preparation of novel peptides represented by the following formula

R₁-A-Tyr(SO₃H)-C-D-Trp-Met-Y

(6)

wherein; R₁ denotes pGlu, X-R₂-CO-, X being carboxyl or amino and R₂ being lower alkylene of 1-6 carbon atoms, or HOOC-CO-; A denotes

20 Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH₂, Asp-NH₂, or Asp-Phe-NH₂; with the proviso that Y is NH₂ or Asp-NH₂ when R₁ is pGlu, HOOC-R₂-CO-, or

25 HOOC-CO-, C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that a sulfo group of a protected peptide amide sulfate ester obtained by the sulfate-ester reaction of the Tyr residue in the protected peptide of the following formula

R₁-A-Tyr-C-D-Trp-Met-Y

(2)

wherein; R₁, A, C, D, and Y are the same as defined above, is converted to a salt of divalent metal such as Ca, Zn, and the like to stabilize said protected peptide amide sulfate ester, and thereafter said protected peptide amide sulfate ester is deprotected.

30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 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2235 2240 2245 2250 2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460 2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520 2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580 2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640 2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700 2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760 2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820 2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880 2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940 2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000 3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060 3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 4170 4175 4180 4185 4190 4195 4200 4205 4210 4215 4220 4225 4230 4235 4240 4245 4250 4255 4260 4265 4270 4275 4280 4285 4290 4295 4300 4305 4310 4315 4320 4325 4330 4335 4340 4345 4350 4355 4360 4365 4370 4375 4380 4385 4390 4395 4400 4405 4410 4415 4420 4425 4430 4435 4440 4445 4450 4455 4460 4465 4470 4475 4480 4485 4490 4495 4500 4505 4510 4515 4520 4525 4530 4535 4540 4545 4550 4555 4560 4565 4570 4575 4580 4585 4590 4595 4600 4605 4610 4615 4620 4625 4630 4635 4640 4645 4650 4655 4660 4665 4670 4675 4680 4685 4690 4695 4700 4705 4710 4715 4720 4725 4730 4735 4740 4745 4750 4755 4760 4765 4770 4775 4780 4785 4790 4795 4800 4805 4810 4815 4820 4825 4830 4835 4840 4845 4850 4855 4860 4865 4870 4875 4880 4885 4890 4895 4900 4905 4910 4915 4920 4925 4930 4935 4940 4945 4950 4955 4960 4965 4970 4975 4980 4985 4990 4995 5000 5005 5010 5015 5020 5025 5030 5035 5040 5045 5050 5055 5060 5065 5070 5075 5080 5085 5090 5095 5100 5105 5110 5115 5120 5125 5130 5135 5140 5145 5150 5155 5160 5165 5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 6245 6250 6255 6260 6265 6270 6275 6280 6285 6290 6295 6300 6305 6310 6315 6320 6325 6330 6335 6340 6345 6350 6355 6360 6365 6370 6375 6380 6385 6390 6395 6400 6405 6410 6415 6420 6425 6430 6435 6440 6445 6450 6455 6460 6465 6470 6475 6480 6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540 6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600 6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660 6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720 6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780 6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840 6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900 6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960 6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020 7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080 7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140 7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200 7205 7210 7215 7220 7225 7230 7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050

thereof, characterized in that a divalent metal salt of a sulfonated p-amino phenylalanine derivative is prepared, and a peptide chain is elongated by use of said divalent metal salt.

13. An accelerator for pancreatic function which contains a novel peptide represented by the following formula



wherein; R_1 denotes pGlu, $X\text{-}R_2\text{-CO-}$, X being carboxyl or amino and R_2 being lower alkylene of 1-6 carbon atoms, or $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$; A denotes Asp, Ala, or merely a chemical bond; B denotes $\text{Tyr}(\text{SO}_3\text{H})$ or $\text{Phe}(\text{NHSO}_3\text{H})$; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH_2 , Asp-NH_2 , or Asp-Phe-NH_2 ; with the proviso that Y is NH_2 or Asp-NH_2 when R_1 is pGlu, $\text{HOOC}\text{-}R_2\text{-CO-}$, or $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$, B is $\text{Tyr}(\text{SO}_3\text{H})$, C is Met, and D is Gly, and pharmacologically acceptable salts thereof, as an effective component.

14. A reagent for pancreatic function tests or a contrast medium for gallbladder which contains a novel peptide represented by the following formula



wherein; R_1 denotes pGlu, $X\text{-}R_2\text{-CO-}$, X being carboxyl or amino and R_2 being lower alkylene of 1-6 carbon atoms, or $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$; A denotes Asp, Ala, or merely a chemical bond; B denotes $\text{Tyr}(\text{SO}_3\text{H})$ or $\text{Phe}(\text{NHSO}_3\text{H})$; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes NH_2 , Asp-NH_2 , or Asp-Phe-NH_2 ; with the proviso that Y is NH_2 or Asp-NH_2 when R_1 is pGlu, $\text{HOOC}\text{-}R_2\text{-CO-}$, or $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$, B is $\text{Tyr}(\text{SO}_3\text{H})$, C is Met, and D is Gly, and pharmacologically acceptable salts thereof as an effective component.

Patentansprüche

1. Neue Peptide der folgenden Formel



worin bedeuten:

R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$;

A Asp, Ala oder lediglich eine chemische Bindung;

B $\text{Tyr}(\text{SO}_3\text{H})$ oder $\text{Phe}(\text{NHSO}_3\text{H})$;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y NH_2 , Asp-NH_2 oder Asp-Phe-NH_2 ;

mit der Massgabe, dass Y die Bedeutung von NH_2 oder Asp-NH_2 hat, wenn R_1 pGlu, $\text{HOOC}\text{-}R_2\text{-CO-}$, oder $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$, B $\text{Tyr}(\text{SO}_3\text{H})$, C Met und D Gly bedeuten, und pharmakologisch annehmbare Salze hiervon.

2. Neue Peptide und Salze nach Anspruch 1, wobei R_1 die Bedeutung von $\text{NH}_2\text{-}R_2\text{-CO-}$ hat, worin R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen ist.

3. Neue Peptide und Salze nach Anspruch 1 oder 2, wobei B die Bedeutung von $\text{Phe}(\text{NHSO}_3\text{H})$ hat.

4. Neue Peptide und Salze nach einem der vorhergehenden Ansprüche, wobei D die Bedeutung D-Ala oder D-Trp hat.

5. Neue Peptide und Salze nach einem der vorhergehenden Ansprüche, wobei Y die Bedeutung NH_2 oder Asp-NH_2 hat.

6. Neue Peptide und pharmakologisch annehmbare Salze hiervon nach einem der vorhergehenden Ansprüche, wobei R_1 die Bedeutung pGlu, $X\text{-}R_2\text{-CO-}$, worin X eine Carboxyl- oder Aminogruppe und R_2 eine niedere Alkyengruppe mit 1-6 Kohlenstoffatomen bedeuten, oder $\text{HOOC}-\text{C}_6\text{H}_4-\text{CO-}$, hat,

A Ala und C lediglich eine chemische Bindung bedeuten.

7. Neues Peptid nach Anspruch 1, gewählt aus:

$\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-NH}_2$,
 $\text{Suc-Asp-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Ala-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Suc-Phe}(\text{NHSO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Gly-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\beta\text{-Ala-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{pGlu-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Pht-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Ala-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Phe}(\text{NHSO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-NH}_2$,
 $\text{Glt-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-NH}_2$,
 $\text{Gly-Asp-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\beta\text{-Ala-Asp-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Ala-Tyr}(\text{SO}_3\text{H})\text{-Gly-Trp-Met-Asp-Phe-NH}_2$, und
 $\text{pGlu-Ala-Tyr}(\text{SO}_3\text{H})\text{-Gly-Trp-Met-Asp-Phe-NH}_2$.

8. Neues Peptid nach Anspruch 1, gewählt aus:

$\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Ala-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Suc-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Suc-Phe}(\text{NHSO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Pht-Tyr}(\text{SO}_3\text{H})\text{-Met-D-Trp-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Gly-Asp-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\beta\text{-Ala-Asp-Tyr}(\text{SO}_3\text{H})\text{-Met-Gly-Trp-Met-Asp-Phe-NH}_2$,
 $\text{Glt-Ala-Tyr}(\text{SO}_3\text{H})\text{-Gly-Trp-Met-Asp-Phe-NH}_2$, und
 $\text{pGlu-Ala-Tyr}(\text{SO}_3\text{H})\text{-Gly-Trp-Met-Asp-Phe-NH}_2$.

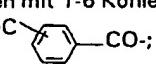
9. Pharmakologisch annehmbares Peptidsalz nach einem der vorangehenden Ansprüche, gewählt aus Alkalimetallsalzen, Erdalkalimetallsalzen, organischen Aminsalzen und Ammoniumsalzen.

10. Verfahren zur Herstellung neuer Peptide der folgenden Formel

$R_1\text{-A-Tyr}(SO_3H)\text{-C-D-Trp-Met-Y}$

(6)

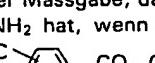
worin bedeuten:

R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder 

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y NH_2 , $Asp\text{-NH}_2$ oder $Asp\text{-Phe-NH}_2$; mit der Massgabe, dass Y die Bedeutung NH_2 oder $Asp\text{-NH}_2$ hat, wenn R_1 pGlu, $HOOC\text{-}R_2\text{-CO-}$ oder 

$HOOC\text{-}R_2\text{-CO-}$, C Met und D Gly bedeuten, und pharmakologisch annehmbare Salze hiervon, dadurch gekennzeichnet, dass der Tyr-Rest in dem Peptid der folgenden Formel

 $R_1\text{-A-Tyr-C-D-Trp-Met-Y}$

(2)

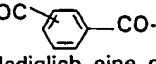
worin R_1 , A, C, D und Y in gleicher Weise wie oben definiert sind, oder dem Peptid, in dem aktive Gruppen, wie etwa Aminogruppen, durch geeignete Schutzgruppen geschützt sind, der Sulfat-Ester-Reaktion unterzogen wird, um den Tyr-Rest in den $Tyr(SO_3H)$ -Rest umzuwandeln.

11. Verfahren zur Herstellung neuer Peptide der folgenden Formel

 $R_1\text{-A-Tyr}(SO_3H)\text{-C-D-Trp-Met-Y}$

(6)

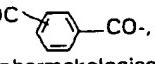
worin bedeuten:

R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder 

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y NH_2 , $Asp\text{-NH}_2$ oder $Asp\text{-Phe-NH}_2$; mit der Massgabe, dass Y die Bedeutung NH_2 oder $Asp\text{-NH}_2$ hat, wenn R_1 pGlu, $HOOC\text{-}R_2\text{-CO-}$ oder 

$HOOC\text{-}R_2\text{-CO-}$, C Met und D Gly bedeuten, und pharmakologisch annehmbare Salze hiervon, dadurch gekennzeichnet, dass eine Sulfogruppe eines geschützten Peptidamid-Sulfatesters, erhalten durch die Sulfat-Ester-Reaktion des Tyr-Restes in dem geschützten Peptid der folgenden Formel

 $R_1\text{-A-Tyr-C-D-Trp-Met-Y}$

(2)

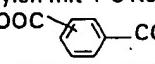
worin R_1 , A, C, D und Y in gleicher Weise wie oben definiert sind, in ein Salz eines zweiwertigen Metalls, wie etwa Ca, Zn und dergleichen überführt wird, um den geschützten Peptidamid-Sulfat-Ester zu stabilisieren und danach der geschützte Peptidamid-Sulfat-Ester von der Schutzgruppe befreit wird.

12. Verfahren zur Herstellung neuer Peptide der folgenden Formel

 $R_1\text{-A-Phe}(NHSO_3H)\text{-C-D-Trp-Met-Y}$

(6)

worin bedeuten:

R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder 

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

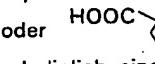
D Gly, D-Ala oder D-Trp, und pharmakologisch annehmbarer Salze hiervon, dadurch gekennzeichnet, dass ein zweiwertiges Metallsalz eines sulfonierten p -Aminophenylalanin-Derivates hergestellt wird und eine Peptidkette durch Verwendung des zweiwertigen Metallsalzes verlängert wird.

13. Beschleunigung für die Pankreasfunktion, enthaltend ein neues Peptid der folgenden Formel

 $R_1\text{-A-B-C-D-Trp-Met-Y}$

(1)

worin bedeuten:

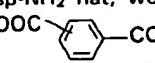
R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1 bis 6 Kohlenstoffatomen bedeuten, oder 

A Asp, Ala oder lediglich eine chemische Bindung;

B $Tyr(SO_3H)$ oder $Phe(NHSO_3H)$;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y NH_2 , $Asp\text{-NH}_2$ oder $Asp\text{-Phe-NH}_2$; mit der Massgabe, dass Y die Bedeutung NH_2 oder $Asp\text{-NH}_2$ hat, wenn R_1 pGlu, $HOOC\text{-}R_2\text{-CO-}$ oder 

B $Tyr(SO_3H)$, C Met und D Gly bedeuten, und pharmakologisch annehmbare Salze hiervon als Wirkstoffkomponente.

14. Reagenz für Pankreasfunktionsprüfungen oder Kontrastmedium für die Gallenblase, enthaltend ein neues Peptid der folgenden Formel

 $R_1\text{-A-B-C-D-Trp-Met-Y}$

(1)

worin bedeuten:

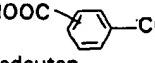
R_1 pGlu, $X\text{-}R_2\text{-CO-}$, worin X Carboxyl oder Amino und R_2 niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder 

A Asp, Ala oder lediglich eine chemische Bindung;

B $Tyr(SO_3H)$ oder $Phe(NHSO_3H)$;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y NH_2 , $Asp\text{-NH}_2$ oder $Asp\text{-Phe-NH}_2$; mit der Massgabe, dass Y die Bedeutung NH_2 oder $Asp\text{-NH}_2$ hat, wenn R_1 pGlu, $HOOC\text{-}R_2\text{-CO-}$ oder 

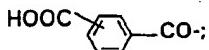
Bedeuten, und pharmakologisch annehmbare Salze hiervon als Wirkstoffkomponente.

Revendications

1. Peptides nouveaux représentés par la formule suivante



dans laquelle R_1 représente pGlu, $X\text{-}R_2\text{-CO-}$, X étant un groupe carboxyle ou amino et R_2 étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Asp, Ala ou simplement une liaison chimique; B représente Tyr(SO_3H) ou Phe(NHSO_3H); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente NH_2 , Asp-NH_2 ou Asp-Phe-NH_2 ; sous réserve que Y représente NH_2 ou Asp-NH_2 lorsque R_1 est un groupe pGlu, $\text{HOOC}\text{-}R_2\text{-CO-}$ ou



B représente Tyr(SO_3H), C représente Met et D représente Gly, et leurs sels pharmacologiquement acceptables.

2. Peptides nouveaux et leurs sels suivant la revendication 1, dans lesquels R_1 représente $\text{NH}_2\text{-}R_2\text{-CO-}$, R_2 étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone.

3. Peptides nouveaux et leurs sels suivant la revendication 1 ou 2, dans lesquels B représente Phe(NHSO_3H).

4. Peptides nouveaux et leurs sels suivant l'une quelconque des revendications précédentes, dans lesquels D représente D-Ala ou D-Trp.

5. Peptides nouveaux et leurs sels suivant l'une quelconque des revendications précédentes, dans lesquels Y représente NH_2 ou Asp-NH_2 .

6. Peptides nouveaux et leurs sels pharmacologiquement acceptables suivant l'une quelconque des revendications précédentes, dans lesquels R₁ représente pGlu, $X\text{-}R_2\text{-CO-}$, X étant un groupe carboxyle ou amino et R₂ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Ala et C est simplement une liaison chimique.

7. Peptide nouveau suivant la revendication 1, choisi entre:

Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-NH₂,
Suc-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-NH₂,
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-NH₂,
Suc-Tyr(SO_3H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,
Suc-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Suc-Phe(NHSO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Gly-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
 β -Ala-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
pGlu-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Pht-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO_3H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,

Glt-Phe(NHSO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO_3H)-Met-Gly-Trp-Met-NH₂,
Glt-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-NH₂,
Gly-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
 β -Ala-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH₂, et
pGlu-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH₂.

8. Peptide nouveau suivant la revendication 1, choisi entre:

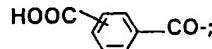
5 Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-NH₂,
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-NH₂,
Suc-Tyr(SO_3H)-Met-D-Ala-Trp-Met-Asp-Phe-NH₂,
Suc-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Suc-Phe(NHSO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Pht-Tyr(SO_3H)-Met-D-Trp-Trp-Met-Asp-Phe-NH₂,
Gly-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
 β -Ala-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH₂,
Glt-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH₂, et
pGlu-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH₂.

10 9. Sel de peptide pharmacologiquement acceptable suivant l'une quelconque des revendications précédentes, choisi entre des sels de métaux alcalins, des sels de métaux alcalino-terreux, des sels d'amines organiques et des sels d'ammonium.

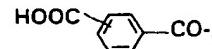
15 10. Procédé de préparation de peptides nouveaux représentés par la formule suivante:



20 dans laquelle R₁ représente pGlu, $X\text{-}R_2\text{-CO-}$, X étant un groupe carboxyle ou amino et R₂ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



25 A représente Asp, Ala ou simplement une liaison chimique; C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente NH_2 , Asp-NH_2 ou Asp-Phe-NH_2 ; sous réserve que Y représente NH_2 ou Asp-NH_2 lorsque R₁ représente pGlu, $\text{HOOC}\text{-}R_2\text{-CO-}$ ou



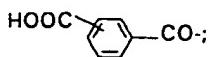
30 Cr représente Met et D représente Gly, et de leurs sels pharmacologiquement acceptables, caractérisé en ce que le résidu Tyr du peptide de formule suivante



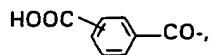
35 dans laquelle R₁, A, C, D et Y sont tels que définis ci-dessus, ou le peptide dont les groupes actifs tels que des groupes amino sont protégés par des groupes protecteurs convenables, est soumis à la réaction d'estérification sulfurique pour convertir le résidu Tyr en résidu Tyr(SO_3H).

40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 1235 1240 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4245 4250 4255 4260 4265 4270 4275 4280 4285 4290 4295 4300 4305 4310 4315 4320 4325 4330 4335 4340 4345 4350 4355 4360 4365 4370 4375 4380 4385 4390 4395 4400 4405 4410 4415 4420 4425 4430 4435 4440 4445 4450 4455 4460 4465 4470 4475 4480 4485 4490 4495 4500 4505 4510 4515 4520 4525 4530 4535 4540 4545 4550 4555 4560 4565 4570 4575 4580 4585 4590 4595 4600 4605 4610 4615 4620 4625 4630 4635 4640 4645 4650 4655 4660 4665 4670 4675 4680 4685 4690 4695 4700 4705 4710 4715 4720 4725 4730 4735 4740 4745 4750 4755 4760 4765 4770 4775 4780 4785 4790 4795 4800 4805 4810 4815 4820 4825 4830 4835 4840 4845 4850 4855 4860 4865 4870 4875 4880 4885 4890 4895 4900 4905 4910 4915 4920 4925 4930 4935 4940 4945 4950 4955 4960 4965 4970 4975 4980 4985 4990 4995 5000 5005 5010 5015 5020 5025 5030 5035 5040 5045 5050 5055 5060 5065 5070 5075 5080 5085 5090 5095 5100 5105 5110 5115 5120 5125 5130 5135 5140 5145 5150 5155 5160 5165 5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 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7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 77

alkylène inférieur de 1 à 6 atomes de carbone, ou un groupe



A désigne un groupe Asp, Ala ou simplement une liaison chimique; C désigne Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente NH₂, Asp-NH₂ ou Asp-Phe-NH₂; sous réserve que Y soit un groupe NH₂ ou Asp-NH₂ lorsque R₁ représente pGlu, HOOC-R₂-CO- ou



C représente Met et D représente Gly, et de leurs sels pharmacologiquement acceptables, caractérisé en ce qu'un groupe sulfo d'un ester sulfurique d'amide peptidique protégé obtenu par la réaction d'estérification sulfurique du résidu Tyr dans le peptide protégé de formule suivante



où R₁, A, C, D et Y sont tels que définis ci-dessus, est converti en un sel d'un métal divalent tel que Ca, Zn, etc. pour stabiliser ledit ester sulfurique d'amide peptidique protégé, puis cet ester est débarrassé de sa protection.

12. Procédé de préparation de peptides nouveaux représentés par la formule suivante



dans laquelle R₁ représente pGlu, X-R₂-CO-, X étant un groupe carboxyle ou amino et R₂ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe

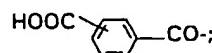


A représente Asp, Ala ou simplement une liaison chimique; C désigne Met ou simplement une liaison chimique; D désigne Gly, D-Ala ou D-Trp, et de leurs sels pharmacologiquement acceptables, caractérisé en ce qu'on prépare un sel de métal divalent d'un dérivé sulfoné de p-aminophénylalanine et on allonge une chaîne peptidique en utilisant ledit sel métallique divalent.

13. Accélérateur pour la fonction pancréatique, qui contient un peptide nouveau représenté par la formule suivante



5 dans laquelle R₁ représente pGlu, X-R₂-CO-, X étant un groupe carboxyle ou amino et R₂ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



10 A représente Asp, Ala ou simplement une liaison chimique; B désigne Tyr(SO₃H) ou Phe(NHSO₃H); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente NH₂, Asp-NH₂ ou Asp-Phe-NH₂; sous réserve que Y soit un groupe NH₂ ou Asp-NH₂ lorsque R₁ représente pGlu, HOOC-R₂-CO-, ou



20 B représente Tyr(SO₃H), C représente Met et D représente Gly, et ses sels pharmacologiquement acceptables, comme composant actif.

14. Réactif destiné à des tests sur la fonction pancréatique ou milieu de contraste pour la vésicule biliaire, qui contient un peptide nouveau représenté par la formule suivante



30 dans laquelle R₁ représente pGlu, X-R₂-CO-, X étant un groupe carboxyle ou amino et R₂ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



40 A représente Asp, Ala ou simplement une liaison chimique; B représente Tyr(SO₃H) ou Phe(NHSO₃H); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente NH₂, Asp-NH₂ ou Asp-Phe-NH₂; sous réserve que Y soit un groupe NH₂ ou Asp-NH₂ lorsque R₁ représente pGlu, HOOC-R₂-CO- ou



50 B représente Tyr(SO₃H), C représente Met et D représente Gly, et ses sels pharmacologiquement acceptables, comme composant actif.

0 132 919

FIG. 1

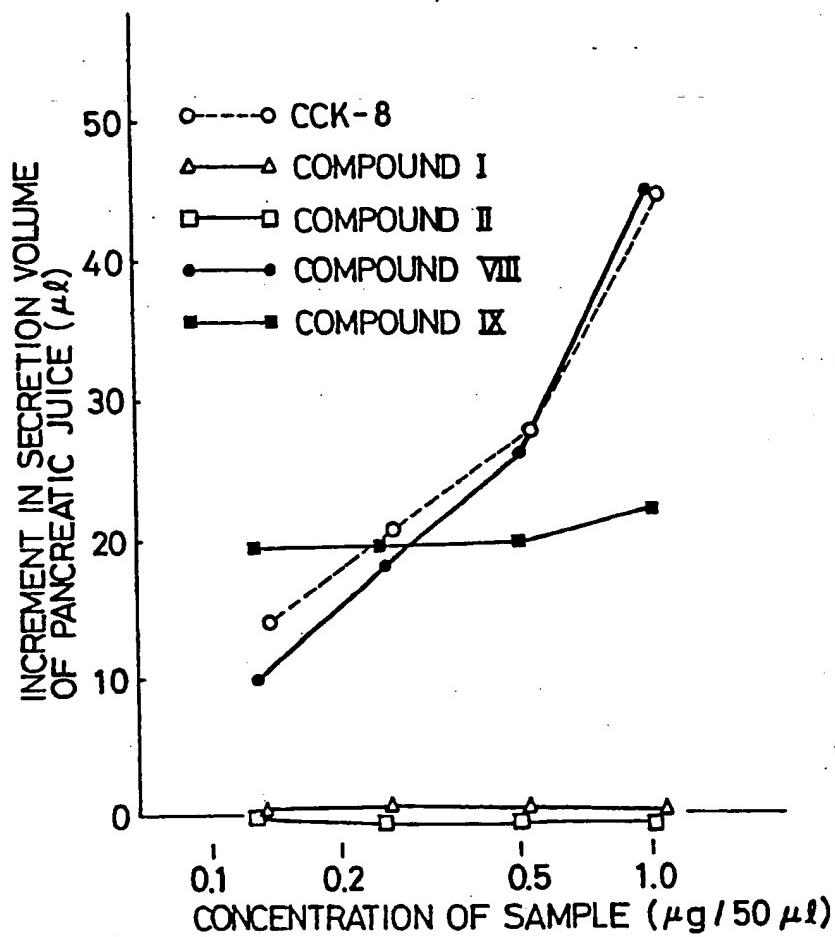


FIG. 2

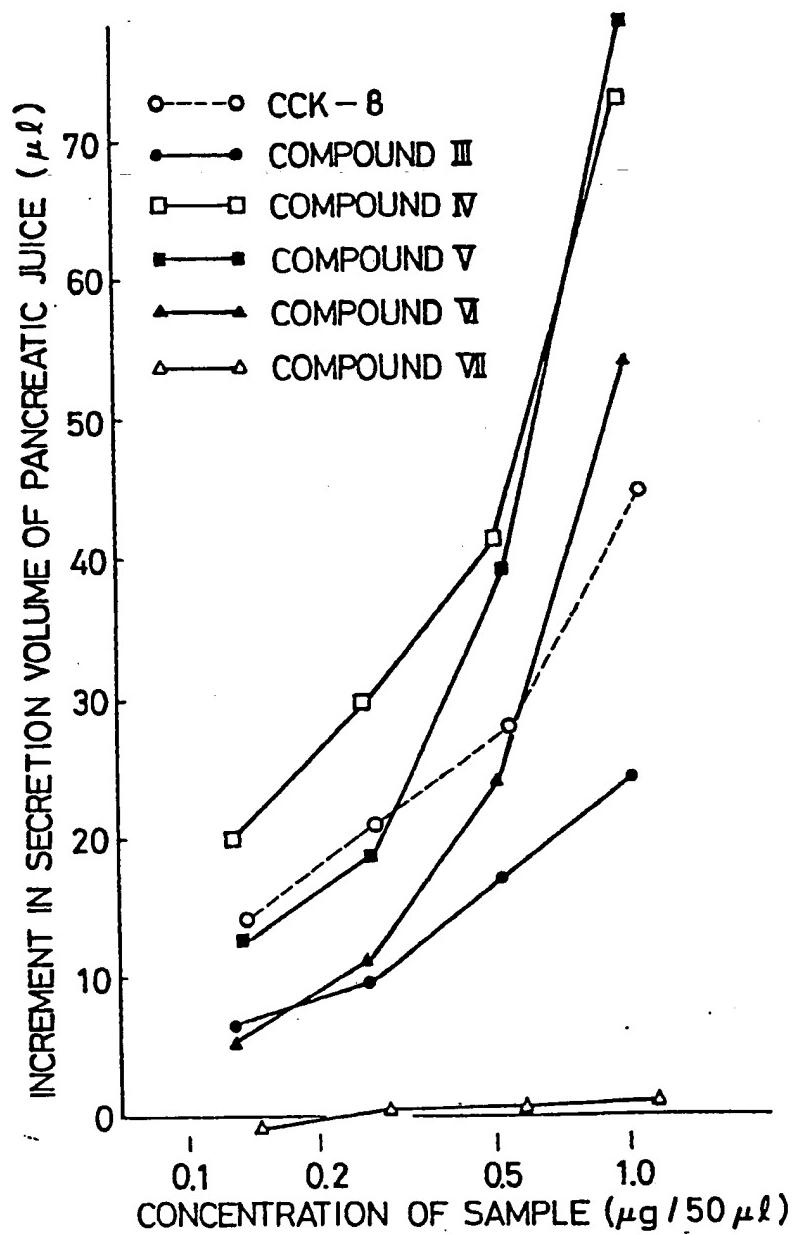
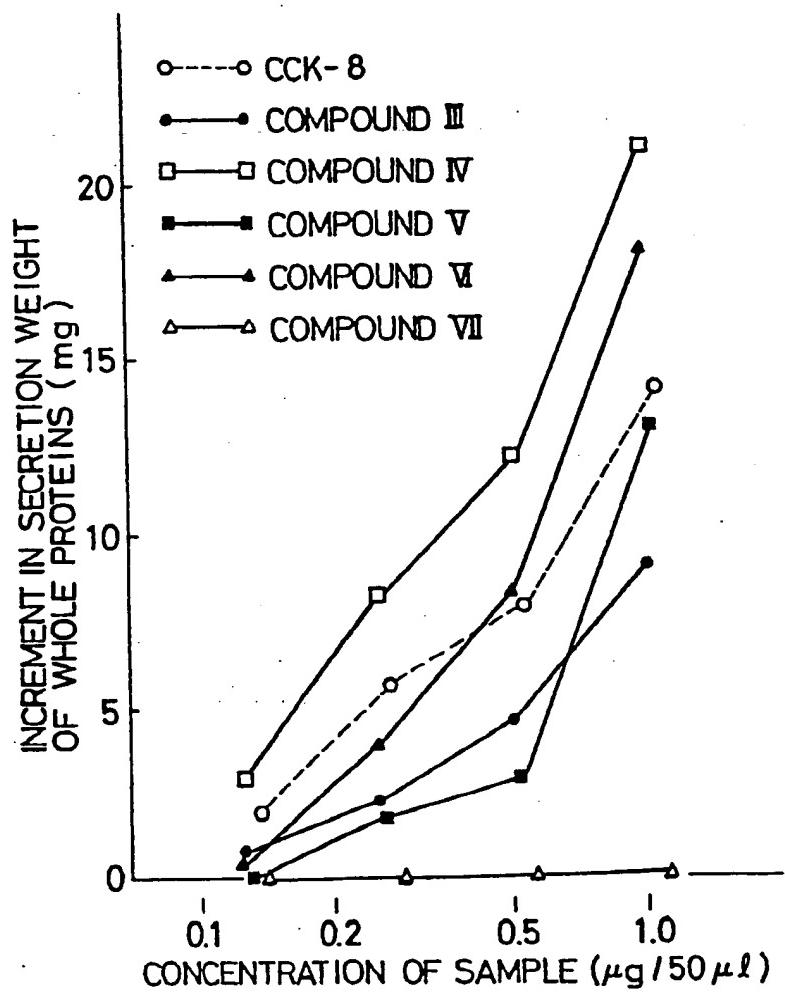


FIG. 3



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FIG. 4

